Abstract No. Tier0486

XAS of Resting State Metallo-beta-Lactamases

P. Crawford (Miami U.), M. Crowder (Miami U.) and D. Tierney (U. New Mexico)

Beamline(s): X9B

Introduction: Bacterial resistance to penicillins and cephalosporins[1,2] is affected by a family of enzymes known as β-lactamases, which hydrolyze the N-C(=O) lactam bond before the antibiotic reaches its target. Approximately 20 of the over 300 known β-lactamases require one or two equivalents of Zn(II) for activity and generally display very broad substrate profiles.[3] The metallo-β-lactamases divide into three classes based on their activity with an intact dinuclear metal site, and based on their quaternary structure. All characterized examples show relatively high (> 70%) activity with a single equivalent of Zn(II).[4] Most of the enzymes show increased reactivity with two metal ions, and these hydrolyze a broader array of β-lactam antibiotics than any of the related serine hydrolases.[3] The known exceptions are three metallo-β-lactamases (two from *Aeromonas*[5,6] and one from *Burkholderia*[7]) that are *inhibited* by the presence of the an intact metal cluster ("2nd-metal inhibited"), and these enzymes display very narrow substrate profiles, much like the serine hydrolase lactamases. A cartoon depicting the general structure of the active sites is shown in Figure 1 (the tetrameric "2nd-metal activated" L1 from *S. maltophila* replaces the cysteine on Zn_2 with a histidine).

The current studies were intended to accomplish two goals. The first was to establish the identity of the active form of the "2nd-metal inhibited" lactamase imiS from *A. sobria*. The second goal was to establish a baseline for time-dependent studies to come. This involved characterization of examples from all three classes of metallo-β-lactamases, in both the native Zn(II) and Co(II)-substituted forms, in their resting state.

The active form of imiS. We examined the active site structure of the "2nd metal inhibited" lactamase imiS with one and two equivalents of Zn(II) present. Fourier transforms of the Zn and Co K-edge EXAFS data are shown in Figure 2. The major peak in the FT for mono-Zn imiS (Figure 2, top) appears at R + α = 1.6 Å, with a shoulder to higher R. These data can only be adequately modeled by the inclusion of a single sulfur donor, consistent with occupation of the Zn_2 site (Figure 1). This result has been verified, and contradicts the previous assumption that the active form of imiS includes a single Zn(II) ion bound at the Zn_1 site.

Resting state β-lactamases. The di-Co derivatives of the β-lactamases are catalytically competent, although they are slower than the di-Zn enzymes.[8] The di-Co derivatives offer the possibility to monitor changes in structure between the native Zn enzymes and their Co-substituted derivatives by other techniques. We have measured preliminary data on the Zn and Co forms of members of all three classes of metallo-β-lactamase. In all three cases, the EXAFS data demonstrate that the Co-for-Zn substitution is structurally valid. In addition, comparison of the mono- and di-substituted datasets show a clear metal-metal interaction in the dinuclear enzymes (see Figure 2), consistent with a Zn-Zn/Co-Co distance of ~ 2.7 Å. In upcoming experiments, we will characterize a mixed-metal Co/Zn version of L1.

Literature Cited

(1) Tripper, D. J.; Strominger, J. L. *Proc. Natl. Acad. Sci.* **1965**, *54*, 1133.
(2) Franceschini, N.; Perilli, M.; Segatore, B.; Setacci, D.; Amicosante, G.; Mazzariol, A.; Cornaglia, G. *Antimicrob. Agents Chemother.* **1998**, *42*, 1459-1462.
(3) Felici, A.; Amicosante, G.; Oratore, A.; Strom, R.; Ledent, P.; Joris, B.; Fanuel, L.; Frere, J. M. *Biochem. J.* **1993**, *291*, 151-155.
(4) Crowder, M. W.; Wang, Z.; Franklin, S. L.; Zovinka, E. P.; Benkovic, S. J. *Biochemistry* **1996**, *35*, 12126-12132.
(5) Rasmussen, B. A.; Bush, K. *Antimicrob. Agents Chemother.* **1997**, *41*, 223-232.
(6) Felici, A.; Amicosante, G. *Antimicrob. Agents Chemother.* **1995**, *39*.
(7) Walsh, T. R.; Gamblin, S.; Emery, D. C.; MacGowan, A. P.; Bennett, P. M. *J. Antimicrob. Chemother.* **1996**, *37*, 423-431.
(8) Crowder, M. W., unpublished results.

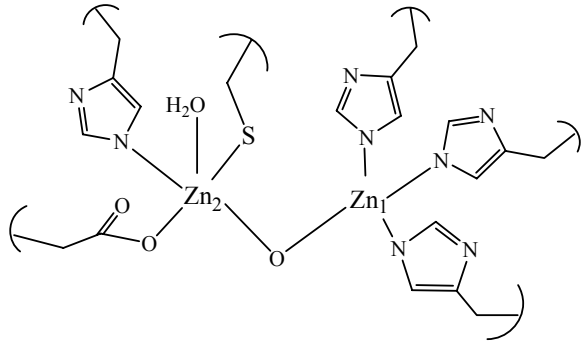

Figure 1. General structure of the metallo-β-lactamase dinuclear active site.

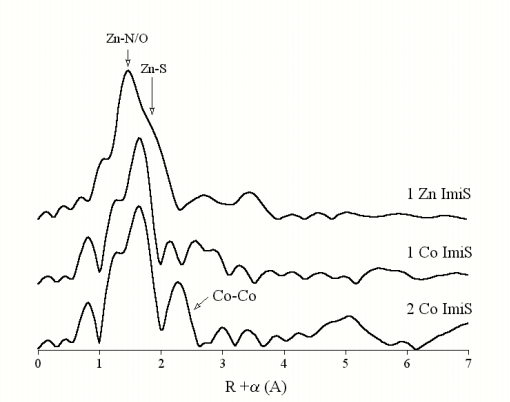

Figure 2. FTs of Zn and Co imiS EXAFS.